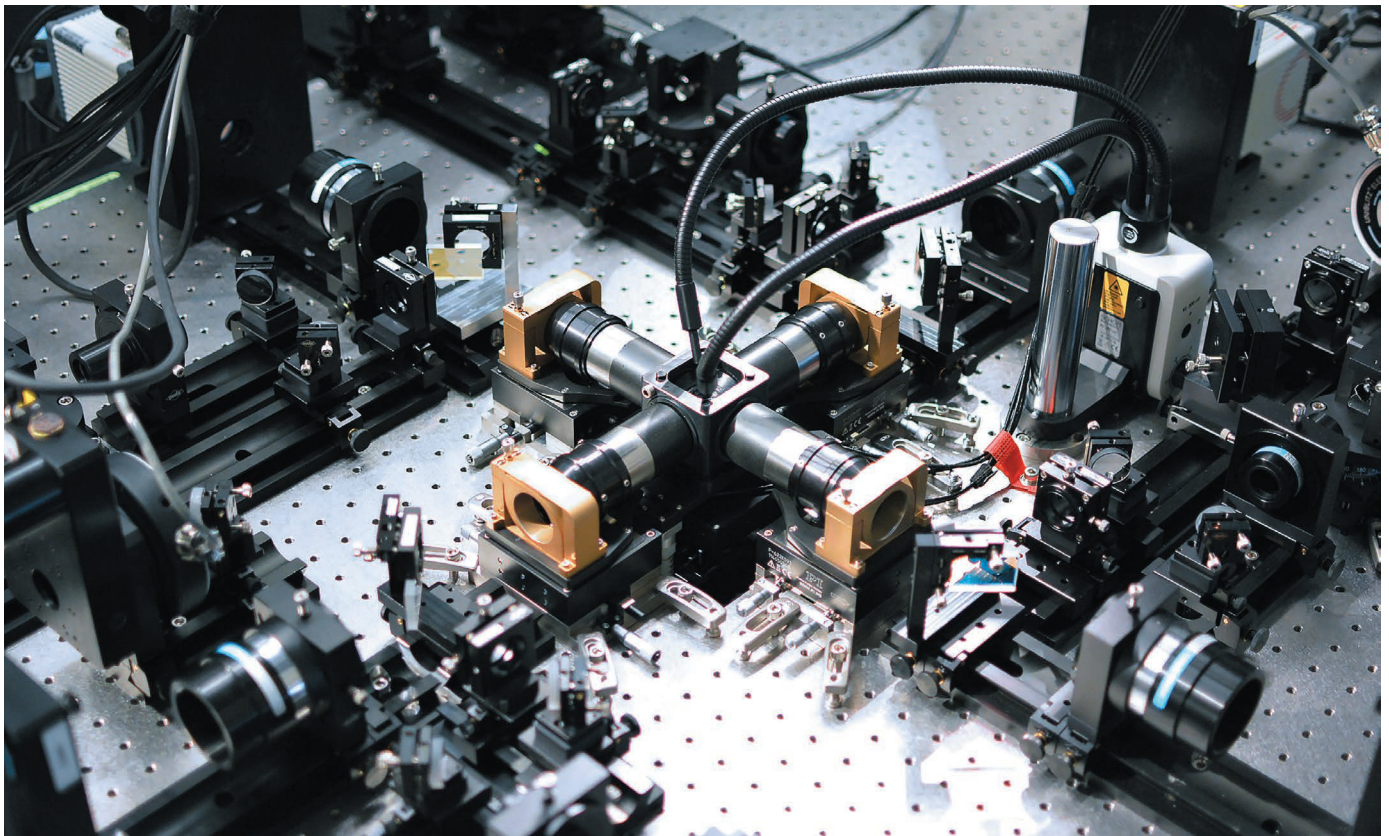


## TECHNOLOGY FEATURE

# THE MICROSCOPE MAKERS

*A small community of scientists has taken a do-it-yourself approach to microscopy: when the right tool for the job doesn't exist, make it.*

RAGHAV CHHETRI/PHILIPP J. KELLER



Custom microscopes, such as the IsoView light-sheet microscope shown here, allow researchers to push beyond the limits of commercial systems.

BY BRIAN OWENS

While pursuing a bioengineering PhD at the University of Pennsylvania in Philadelphia, Wesley Legant ran into a frustrating roadblock: he had ideas, but the equipment to carry them out didn't yet exist.

With an interest in cell mechanics and motility, Legant was developing tools to measure the forces that cells exert on their environment. He embedded fluorescent beads in the material surrounding a growing mammalian cell so that as the cell moved, it would deform the material, moving the beads. By measuring how much the beads moved, Legant could calculate the forces exerted by the cell<sup>1</sup>. Still, he had difficulty getting

accurate data. “The tools were successful, but I was quickly coming up against limitations in available microscopes,” he says.

Cells move slowly under their own power — the fastest creep along at a few micrometres per minute — so the microscopes need to watch the action for a long time. And to track the beads in 3D, Legant had to image the entire volume at high spatial resolution. This was in the late 2000s and early 2010s, and the commercial microscopes available at that time — point-scanning and spinning-disk confocal microscopes — weren't up to the job.

“Both of those techniques actually had enough resolution to do the type of tracking that we wanted to do, but they were far too

phototoxic and too slow,” Legant says.

Picture a transparent cube. Confocal microscopes allow scientists to capture every point in the cube one by one, and gradually build up a 3D image. To do so, they project a beam of light vertically through the sample, illuminating a column extending through the cube at each point. But each flash of light generates reactive oxygen species that damage the sample — the ‘phototoxic’ effect to which Legant is referring. At the same time, the light-emitting ‘fluorophores’ detected by the microscope fade over time, through a process called photobleaching.

In Legant's experiments, each 3D image took about a minute to acquire. He then had to wait another five minutes to take the next ▶

► picture, to give the cells time to recover in between and so avoid killing them before the desired data could be gathered. He was able to measure the forces exerted by the cells, but not at the level of detail he wanted.

To tackle those problems, Legant switched focus during his postdoctoral research. Working with physicist and microscopy specialist Eric Betzig at the Howard Hughes Medical Institute's Janelia Research Campus in Ashburn, Virginia, he joined the small but growing do-it-yourself (DIY) microscopy community.

### LIVE IMAGING

Building microscopes is a complex and time-consuming challenge, and it requires a team with the right mix of skills to handle the array of optical, mechanical and computer parts involved. But the rewards can be huge. A new microscope can advance the science not just of biology, but of microscopy itself.

At Janelia, researchers push the boundaries of neuroscience and developmental biology. Those are fields that rely heavily on microscopy and imaging, and the institute has plenty of off-the-shelf commercial microscopes. But when the tools they need don't exist, they don't wait for them to be invented elsewhere — they build them in-house.

“What motivates our work in tech development is that we want to enable new types of experiments that we simply can't do with existing microscopes,” says Philipp Keller, a physicist at Janelia who studies nervous-system development in zebrafish and fruit flies.

In the mid-2000s, while working at the European Molecular Biology Laboratory in Heidelberg, Germany, Keller faced a problem similar to Legant's: he wanted to track all the cells in a developing zebrafish embryo to learn how they move and coalesce to form different tissues and organs. But most existing microscopes could not image a specimen of that size — a ball of cells about 700 micrometres across — over a long period of time without also killing it as a result of the intense lighting required.

So, Keller turned to light-sheet microscopy, a technique that was just coming into use at the time. Rather than illuminating the entire sample, light-sheet microscopes project a weakly focused, low-intensity ‘sheet’ of light directly into the focal plane of the object that the user wants to image. A high-quality camera can capture the entire focal plane in a single exposure, and by moving that plane vertically through the sample, researchers can reconstruct the entire 3D object.

“Light-sheet microscopy is a very fast imaging technique, but it is also very gentle,” says Keller. “There are no out-of-focus structures being exposed [to] and potentially damaged by light.” At the time, no existing off-the-shelf microscope was suitable for Keller's research so, in 2005, he decided to build one<sup>2</sup>. His design, called a Digital Scanned Laser Light Sheet Fluorescence Microscope (DSLM),

could capture every cell in a developing zebrafish embryo in 90 seconds.

Not all microscope builders have a specific research question in mind when they begin designing. For Stefan Hell, a physicist at the Max Planck Institute for Biophysical Chemistry in Göttingen, Germany, coming up with new concepts in microscopy is a goal in itself. “It's a science project. I'm doing it for the sake

## Ten steps to a DIY microscope

Building a microscope is complicated, but Philipp Keller, a physicist at the Howard Hughes Medical Institute's Janelia Research Campus in Ashburn, Virginia, has distilled the process down to ten steps:

- Brainstorm the instrument.
- Plan and test the optical design.
- Use computer-aided design software to engineer the body and custom pieces.
- Order parts, and fabricate customized mechanical and optical components.
- Borrow components to test their performance and ease of integration.
- Assemble the prototype.
- Code the microscope control software.
- Refine custom components on the basis of performance.
- Carry out proof-of-principle experiments.
- Develop and refine the image-processing software. **B.O.**

of coming up with an idea that pushes the art of microscopy,” he says.

And he has been wildly successful at it. In 2014, Hell shared the Nobel Prize in Chemistry with William Moerner of Stanford University in California, as well as Betzig, for the invention of super-resolved fluorescence microscopy, a technique that allows researchers to image biological structures at the nanoscale.

Although Hell has built and commercialized several microscope designs, few biologists ever need to follow suit, he says, especially because commercial manufacturers are so quick to jump on new ideas. His own Nobel-prize-winning design<sup>3</sup>, called stimulated emission depletion, or STED, cost him US\$200,000 to build in 1999. Now, it's available as a push-button system that is half the size of a shoebox and attaches to any existing confocal fluorescence microscope. And there are several companies out there manufacturing light-sheet microscopes as well, he says.

Keller, however, says that the field of light-sheet microscopy is still relatively young, and

that the few commercially available systems are “seven to eight years” behind the “bleeding edge” of scientific research. “We are really forced to build our own.”

The process entails both pros and cons. A custom system can be years ahead of the curve in terms of speed and resolution, and may be customized to focus on specific biological problems or systems. But that comes at the cost of flexibility. In some DIY systems, it can be difficult to do something as simple as changing the magnification. And custom systems require valuable time and effort to set up and maintain.

For those willing to take on the challenge, the rewards are worth it, says Clare Waterman, a cell biologist at the US National Heart, Lung, and Blood Institute in Bethesda, Maryland. In the early 1990s, Waterman exploited new camera technology to develop a technique called fluorescent speckle microscopy<sup>4</sup>, which allows for the study of the cytoskeleton and other large multiprotein complexes. “The advantage is that you get an answer that nobody else can get yet,” she says. “The disadvantage is you have to work out all the bugs yourself. But that's fun!”

### DIY GUIDE

Whether for advancing microscopy or answering a specific biological question, the process of building a new microscope is largely the same. Keller has become so experienced at it — “not a single year has passed where we're not working on a new microscope”, he says — that he has refined the process down to its essentials (see “Ten steps to a DIY microscope”). A good microscope team, Keller says, should include a physicist or biomedical engineer to run the project, supported by at least four specialists: an optical engineer to plan the optical layout; a mechanical engineer to work out how the pieces all fit together; a software developer to program the machine; and a computer scientist to convert raw data into usable images.

The first step is optical design. Using specialized software — Keller and Legant use OpticStudio, available from Zemax in Kirkland, Washington — the optical engineer works in virtual space to define the correct arrangement of lasers, lenses, mirrors and other optical components that will provide the resolution and features required.

The mechanical engineer then works out how all those parts will actually fit together in the real world, as physical pieces bolted to an optical table. “At this point, it's just a bunch of lenses in a line, floating in space,” says Brian Coop, a mechanical engineer who works at Janelia with Keller. “It's up to me to make it stand on its own two feet.”

The biggest challenge at this stage is working within the extremely tight physical constraints on the work, Coop says. When a microscope must focus on things that are just a few micrometres or even nanometres in size, there is little room for error. Lenses, mirrors and lasers need to be held in precise alignment to produce



useful, in-focus images, and Coop needs to take into account how tiny changes, such as the thermal expansion of metals, could throw off the alignment. “Paying attention to the accuracy of the optical alignment makes everything that comes after simpler,” says Coop.

Coop builds as much of the microscope as he can with off-the-shelf parts, or by reusing parts from previous builds. But every microscope has at least a few custom-made pieces, which Coop has to design and sometimes manufacture himself in Janelia’s machine workshop.

The sample chamber in Keller’s latest microscope, for example, has ports to accommodate four objective lenses that are dipped into a medium in which a sample has been submerged. The microscope requires seals that prevent leakage but also allow the lenses to move independently. And because the objectives are so close together, with as little as 100 micrometres of clearance, and all with different sizes and shapes, Coop has to adapt the chambers and seals to accommodate every possible combination. Designing and making each chamber takes two to three days and costs between US\$800 and \$1,000, he estimates.

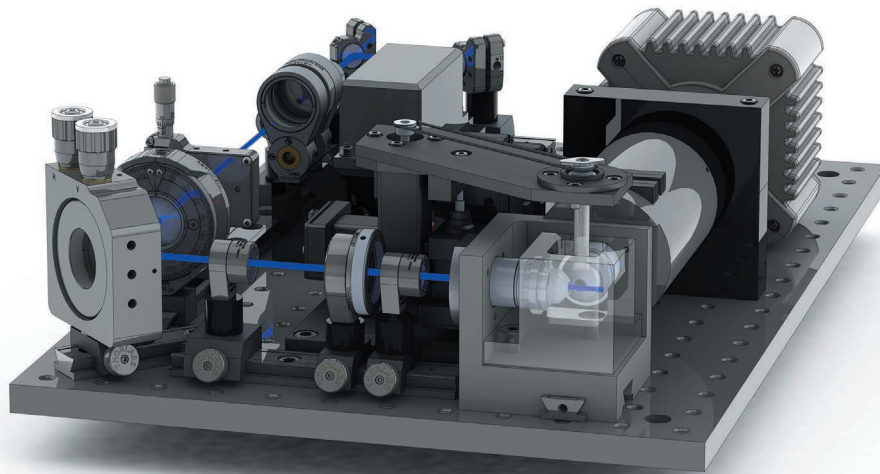
Once the optical and mechanical engineers have assembled a prototype, the software developer and computer scientist jump in to ensure that the parts work together properly and will produce usable images. Many microscope builders use a commercial software package called LabVIEW to control their microscopes, but when machines get more advanced, a custom solution is sometimes needed, says Daniel Milkie, a computer programmer at Janelia.

“We’re designing new tools and new types of microscopes that are pushing the limits of what the hardware is capable of, so you need to have software designed for that to get maximum performance,” he says. The trick is making sure that the software is flexible enough to be quickly adjusted to meet new requirements, such as a greater number of detectors. So, Milkie made the code modular, meaning that it is easy to integrate new elements without having to start from scratch.

But the biggest challenge of the software side, says Milkie, is working out how to deal with the huge volumes of data that the microscopes generate. High-speed cameras can produce a gigabyte of data per second, and some machines have several cameras running at once. The Betzig lab alone can generate 50–100 terabytes of data a year, says Milkie. “We’ve created this firehose, so where does it go?” he says.

The finished product looks nothing like a conventional microscope. All the parts — mirrors, lenses, lasers, cameras and sample chambers — are attached to various posts and clamps across a table weighing several tonnes and designed to insulate the microscope from

**“We want to enable new types of experiments that we can’t do with existing microscopes.”**



Researchers can adapt custom designs, such as those at OpenSPIM.org, into various configurations.

vibrations. It’s like an elaborate Lego kit, says Legant.

Keller estimates that building a microscope from scratch takes at least a year, although that can be reduced if the team can recycle parts and software from an earlier-generation instrument. And because the designs need increasingly advanced customization, development is getting more expensive. Keller’s DSLM cost around \$50,000 to build in 2005, whereas later machines cost \$100,000–200,000. His latest build in 2015 — the isotropic multiview microscope<sup>5</sup> — cost upwards of \$1 million. “I don’t think we’ll ever see the days again where we build a \$50,000 microscope and say that this is an improvement over the state of the art,” Keller says.

#### CUSTOM CONSIDERATIONS

Custom machines also take a little more finesse to use, because they often involve substantial set-up and calibration for each experiment — the kind of hands-on tweaking by the user that commercial manufacturers try to avoid. But Waterman says that this should not be a barrier. “It’s the fundamentals you should learn in a basic microscopy course,” she says.

Published studies involving novel microscopy systems often include plans and parts lists. For those who want a little hand-holding, Janelia makes the plans and software for its microscopes freely available online and provides help with the construction process. “There’s about 20 hours of video tutorials on how to assemble and align everything,” says Legant. And there are other sources of expertise, as well. Sites such as diSPIM.org, from biophysicist Hari Shroff of the US National Institute of Biomedical Imaging and Bioengineering Section on High Resolution Optical Imaging in Bethesda, Maryland, OpenSPIM.org, which started in Pavel Tomancak’s developmental-biology lab at the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden, Germany, and OpenSpinMicroscopy, led by Emilio Gualda at the Institute of Photonic Sciences in Barcelona, Spain, all offer plans for various light-sheet microscope configurations at no cost.

But although building a microscope from

existing plans is simpler than designing one from the ground up, it still requires a knowledge of optics, mechanics, electronics, computer programming and biology. The big advantage is the price, Gualda says. Commercial versions of the selective-plane illumination microscope offered by OpenSpinMicroscopy cost around \$200,000. Using his open-source software and inexpensive hardware such as Arduino controllers, Gualda estimates, researchers can build a high-quality machine for about one-quarter of that price, with the bulk of the cost coming from the laser and camera. “And you can customize it for your own needs,” adds Gualda.

There also are online forums where users can get advice and trade tips. According to Srigokul Upadhyayula, a molecular biologist at Harvard Medical School in Boston, Massachusetts, who worked alongside Legant to build the first lattice light-sheet microscopes in 2014 at Janelia, this sort of collaboration represents a big change in how these scientists usually work. “It’s rare to see in this kind of community — everyone used to be isolated.”

As for Legant, he is now preparing to establish his own lab at the University of North Carolina in Chapel Hill. The position will allow him to continue his work on both cell biology and microscope design. One of his first projects will be to revisit the question of how cells move. “We’ve solved the technical problem with our latest system — we just haven’t had a chance to apply it towards that particular question,” he says. Now that he has created the tools needed to do the work, Legant might finally get the answers he has been chasing for years. ■

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1. Legant, W. R. *et al.* *Nature Methods* **7**, 969–971 (2010).
2. Keller, P. J., Schmidt, A. D., Wittbrodt, J. & Stelzer, E. H. K. *Science* **322**, 1065–1069 (2008).
3. Klar, T. A., Jakobs, S., Dyba, M., Egner, A. & Hell, S. W. *Proc. Natl Acad. Sci. USA* **97**, 8206–8210 (2000).
4. Salmon, E. D. & Waterman, C. M. *Mol. Biol. Cell* **22**, 3940–3942 (2011).
5. Chhetri, R. K. *et al.* *Nature Methods* **12**, 1171–1178 (2015).